Exhibit C

TUMOR VACCINATION

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An autologous whole-cell vaccine has been shown to induce DHR to the whole-cell component, as well as significant regression of metastasis in patients with metastatic malignant melanoma.

Tumor vaccination is an active, specific immunotherapy for malignant disease. It may be defined as "the administration of tumor cells, modified tumor cells, or tumor-cell surface-membrane preparations to stimulate or to augment various components of antitumor immunity to induce tumor regression or to prolong tumor remission achieved by conventional therapy."[1]

Vaccines also may be considered a type of biologic response-modifier therapy. The approach is based on the belief that the host is capable of mounting an effective immune response against tumors if appropriately stimulated, a belief that was first advanced around the turn of the century.[2] The concept of immunologic surveillance, which evolved some 40 to 50 years later, suggested that the human host was capable, under certain circumstances, of rejecting a tumor essentially in the same manner as a homograft was rejected.[3]

The first attempt to vaccinate humans against cancer was undertaken in 1902.[4] In this initial attempt, fluid was extracted from tumors in patients with advanced disease.[5] Over the next 50 years, a great variety of tumor cell preparations obtained from autologous or allogeneic tumors were used, generally to treat patients with advanced disease.

Fresh interest in the clinical potential of tumor vaccination was stimulated in the 1950s and 1960s by experimental studies conducted in syngeneic rodents. These demonstrated unequivocally that chemically induced and virally induced tumors had both shared and uniquely individual tumor-specific transplantation antigens (TSTAs).

Humoral and cellular immune responses were shown to exist in patients with cancer; these were found to be directed against tumor-associated antigens (TAAs) rather than against TSTA. Also, TAAs were found on embryonic cells and tumor cells. The "unique antigens" on human tumor cells appear to result from tumor cell dedifferentiation for display of a partial embryonic-cell-membrane antigenic profile. Other TAAs arise as a consequence of the modification of normal "self" antigens producing an "altered self" phenotype.

TABLE 1

Antitumor Immune Mechanisms

- Activated macrophage cytotoxicity
- Cytotoxic T cells
- Natural killer (NK) cells
- Lymphokine-activated killer cells
- Humoral antibodies (complement dependent)
- Antibody-dependent cellular cytotoxicity (complement-independent, macrophage, neutrophil or NK-cell-dependent)

MANY UNRESOLVED CLINICAL ISSUES

The principal human immune responses to tumor antigens are listed in Table 1. It has not been definitively established which of these immune responses, alone or in combination, are the most important in a host response to cancer, nor is it clear which should be targeted for stimulation with tumor vaccination. There are many unresolved issues pertaining to the actual vaccine formulation that need to be addressed; some of these depend on whether the vaccine formulation is based on intact tumor cells (Table 2) or tumor cell extracts or products (Table 3).

In addition to vaccine formulation, several other questions remain:

- Should cellular extracts or whole cells be mixed with immunomodulating adjuvants

to increase tumor vaccine immunogenicity?

- Should tumor vaccines with or without adjuvants be used alone or in combination with cytotoxic drugs that can modulate or suppress undesirable immune responses?
- Should cytokines be used to augment immune responses to a vaccine?
- Is there a place for tumor vaccination in patients with advanced cancer?
- What is the appropriate dose, schedule, and route of administration for effective tumor vaccination?
- What measurement or surrogate biologic end point can be used to assess the biologic effectiveness of the vaccine?
- Will the immune response against TAA produced by a human tumor vaccine be selective and specific for tumor cells or will autoimmune reactions against normal cells be a possible toxicity associated with vaccination?

Advances in molecular genetics and the availability of monoclonal antibody reagents now make it possible to purify cells and cell components with defined and unique antigenic characteristics for use in human tumor vaccines. However, a number of the promising clinical trials of tumor vaccination conducted in the 1970s and 1980s used relatively simple and empiric methods of tumor vaccination preparation.

In one study, surgically resected stage I and II lung cancer patients were treated with a vaccine prepared from allogeneic tumor cells.[6] Cell membranes from viable tumor cells were subjected to low-frequency sonication and the soluble material separated with Sephadex G-200. Polyacrylamide gel electrophoresis was used to purify protein band material, which could elicit delayed hypersensitivity reactions (DHRs) in lung cancer patients. This material was administered intracutaneously in combination with Freund's complete adjuvant (FCA) in a series of three injections at monthly intervals beginning about 1 month after surgery.

Pilot studies suggested that this form of therapy delayed or prevented tumor recurrence. The approach was tested in a large multicenter clinical trial, which found no difference in survival between control patients and patients treated with FCA alone or with FCA and tumor antigen.[6] No autoimmune toxicity was noted during the course of these studies. Peripheral blood monocytes producing excessive amounts of prostaglandins appeared in the circulation prior to clinical relapse in patients who failed in all three arms of the study.

Further analysis of this clinical trial, however, suggests that a survival benefit may have been obtained in the fraction of patients in whom careful attention was paid to thorough homogenization of tumor antigen in the FCA. Vaccinated long-term survivors also may have developed more intense DHRs to tumor antigen. The methods employed in this study, although important and innovative, need to overcome the problematic nature of the technique's purification process and reproducibility (Table 3) before wider application in humans is feasible.

A tumor vaccination study in patients with surgically resected Dukes B_2 through C_2 colorectal cancer was conducted, based on rigorously evaluated preclinical experimental animal data in which requirements for effective immunotherapy were established.[7] An elegant series of studies of a guinea pig line-10 hepatocarcinoma model showed convincingly that bacillus Calmette-Guérin (BCG) admixed with syngeneic tumor cells could induce sufficient systemic immunity to elimi-

TABLE 2

Critical Issues for Whole-Tumor-Cell Vaccines

Should autologous or allogeneic cells be employed?
Should cells be obtained from fresh surgical specimens or from tissue cell lines?
Should cells first be irradiated to maintain their membrane integrity but prevent their proliferation?
How can the reproducibility of vaccine preparation be assured?
How can whole tumor cells be used that are gene modified for the following phenotypic changes (individual or in combination) to enhance immunogenicity: (1) expression of HLA class I or II antigens and/or adhesion molecules; (2) secretion of immunomodulatory stimulating cytokines, such as interleukin-2 or tumor necrosis factor; and (3) secretion of chemotactic cytokines?

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nate a limited metastatic disease burden.[8] These studies controlled for variables such as the number and viability of tumor cells, and ratio of viable BCG organisms to tumor cells. In the pilots that evolved from these trials, patients were randomized to a control arm or were vaccinated with their own tumor cells, obtained from surgical specimens at the time of operation and cryopreserved until thawed and irradiated prior to use.[9] Treated patients underwent a schedule of three intradermal vaccine treatments weekly beginning 4 to 5 weeks after tumor resection.

The first two vaccine preparations consisted of irradiated cells and BCG; the third vaccine preparation was composed of irradiated tumor cells alone. Vaccinated patients developed augmented DHR to their autologous tumor cells with greater frequency than nonvaccinated patients. A DHR increase to autologous normal intestinal mucosa cells was not seen. An Eastern Cooperative Oncology Group trial is now evaluating this approach for surgically resected Dukes B_2 and B_3 patients.

FIRST TRIALS WITH HUMAN TUMOR VACCINES

These studies, the first truly large, randomized, controlled, multi-institutional clinical trials of human tumor vaccines for solid tumors, are examples of the use of whole cells and cell extracts for human tumor-vaccine preparation. In each case, an adjuvant substance was added to enhance the immunogenicity of the vaccine.

An autologous whole-cell vaccine has been shown to induce DHR to the whole-cell component, as well as significant regression of metastasis in patients with metastatic malignant melanoma.[10] Patients received cyclophosphamide before the vaccine in an attempt to modulate the activity of suppressor T-lymphocytes. The vaccine was prepared by methods similar to those previously described[7] and combined with BCG.

Other investigators have also used cyclophosphamide to inhibit suppressor T-lymphocyte activity prior to the administration of a malignant melanoma vaccine.[11] The vaccine preparation consisted of mechanically disrupted allogeneic tumor cells from melanoma cell lines. The concentration of vaccine was standardized in the preparation through measurements of a melanoma-associated antigen.

Measurement was performed by binding inhibition enzyme immunoassays using a monoclonal antibody. The vaccine was given subcutaneously with an adjuvant consisting of detoxified endotoxin (monophosphoryl lipid A) mycobacterial cell wall skeleton and squaPane oil. In this trial, regressions of disease were seen in patients with metastatic disease.

VIRAL ONCOLYSATES FOR HUMAN TUMOR VACCINATION

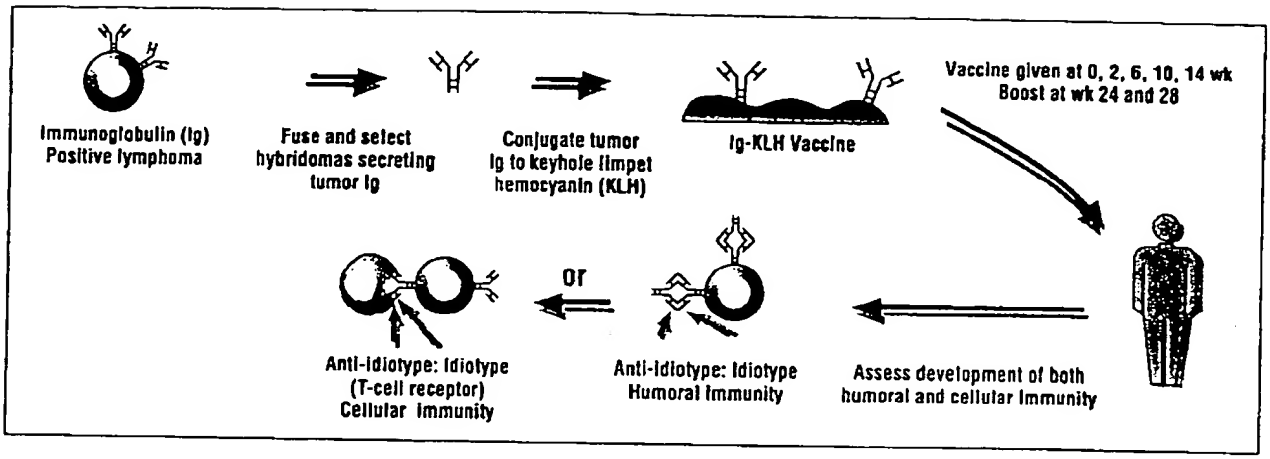
The use of viral oncolysates for human tumor vaccination combines the potential immunogenic benefit of whole cells with the value of cell extracts.[12] Viral oncolysates are homogenates of virus-infected cells. The virus in the mixture is believed to have an adjuvant rather than an antigenic role. Allogeneic and autologous viral oncolysates have been used in human immunotherapy. Influenza virus and vaccinia virus have been most frequently used in the preparation of viral oncolysates since the first report of this procedure in 1974.[13]

Pilot studies have suggested a protective or therapeutic benefit for viral oncolysates in gynecologic cancer, melanoma, and sarcoma. However, these reports must be considered anecdotal until larger randomized investigations are conducted.

TABLE 3

Critical Issues for Tumor-Cell-Extract Vaccines

What method of antigen extraction should be employed?
Should material be derived from a single source or should pooled material from a number of sources be used?
What methods are to be used to identify material in cellular extracts that will produce the most effective stimulation of antitumor immune responses?
Should extracted material be separated from HLA antigens that are present on both normal and malignant cells?
How can reproducibility of vaccine preparation be assured?



It may be possible to vaccinate against some human cancers by immunizing against those few viruses presently known to be associated with cancer in humans. Hepatitis B virus infection is associated with the development of primary hepatocellular cancer. Immunizing against this virus will prevent its hepatic damage and may reduce the incidence of associated cancer.[14]

Finally, one of the most innovative approaches to tumor vaccination that has been developed relies on the use of idiotypic molecules that reiterate the molecular configuration of tumor-associated antigens. This approach is based on principles that predict that the variable regions of immunoglobulins and T-cell receptors that are responsible for antigen recognition are themselves capable of provoking both B-cell and T-cell immunity.[15]

These concepts led other investigators to vaccinate patients who have B-cell lymphoma with the autologous immunoglobulins from each patient's tumor following cytotoxic chemotherapy[16] (Figure). Vaccinated patients developed either humoral immunity, cellular immunity, or both; in the two patients with measurable disease, complete tumor regression was observed. These preliminary results demonstrate the feasibility of idiotypic vaccination for B-cell and T-cell malignant diseases and suggest that similar approaches might also be developed for nonlymphoreticular malignancies.

FIGURE

Strategy for idiotype vaccination. Source: Adapted from Kwak LW, Campbell MJ, Czerwinski DK, Hart S, Miller R, Levy R. Induction of immune responses in patients with B cell lymphoma against surface immunoglobulin idiotype expressed by their tumors. *N Engl J Med.* 1992; 327:1209-1215.

REFERENCES

1. Hersh EM, Marligit GM, Gutteman GM, and Richman SP. Immunotherapy of human cancer. In: Becker FF, ed. *Cancer: A Comprehensive Treatise.* New York, NY: Plenum Press; 1977; 425.
2. Ehrlich P. (1909) The collected papers of Paul Ehrlich. In: Himmelweit F, ed. *Immunology and Cancer Research.* London: Pergamon Press; 1957:550.
3. Burnet M. *Cellular Immunology.* Cambridge, England: Cambridge University Press; 1969.
4. Von Leydon E, Blumenthal F. Vorlaufige mitteilunger Uber einige ergebenisse der krebsforschung auf der I. Medizinischen Klinik Dtsch. *Med Wochenschr.* 1902;28:637-638.
5. Southam CM. Applications of immunology to clinical cancer: past attempts and failure possibilities. *Can Res.* 1961;21:1302-1316.
6. Stewart THM, Shelley WE, Willan AR, Hollinshead AC. An evaluation of the role of tumor-specific antigens. In: Mountain C, ed. *Lung Cancer: Current Status and Prospect for the Future.* Houston, Texas: University of Texas Press; 1986: 351.
7. Hoover HC, Hanna MG. Immunotherapy by active specific immunization: clinical applications. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Biological Therapy of Cancer.* Philadelphia, Pa: JB Lippincott Company, 1991.
8. Hoover HC, Peters LC, Brandhorst JS, et al. Therapy of spontaneous metastases with an autologous tumor vaccine in a guinea pig model. *J Surg Res.* 1981;30:409-415.
9. Hoover HC, Surdyke MG; Dangel RB, et al. Prospectively randomized trial of adjuvant active-specific immunotherapy for human colorectal cancer. *Cancer.* 1985;55:1236-1243.
10. Berd D, Maguire HC Jr, Mastrangelo M. Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. *Can Res.* 1986;46:2572-2577.
11. Mitchell MS, Harel W, Kempf RA, et al. Active-specific immunotherapy for melanoma. *J Clin Oncol.* 1990;8:856-869.
12. Ioannides CG, Platsoncas CD, Patenia R, et al. T-Cell functions in ovarian cancer patients treated with viral oncolysates. Increased helper activity to immunoglobulins production. *Anticancer Research.* 1990;10:645-654.
13. Sinkovics JG, Williams DE, Campos LT, et al. Intensification of immune reactions of patients to cultured sarcoma cells: attempts at monitored immunotherapy. *Semin Oncol.* 1974;1:351-365.
14. Stevenson FK. Tumor vaccine. *FASEB J.* 1991;5:2180-2257.
15. Schwartz RS. Therapeutic clonotypic vaccines. *N Engl J Med.* 1992; 327:1236-1237.
16. Kwak LW, Campbell MJ, Czerwinski DK, et al. Induction of immune responses in patients with B-cell lymphoma against the surface immunoglobulin idiotype expressed by their tumors. *N Engl J Med.* 1992;327:1209-1215.

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